Exhibit L
Part 1 of 3

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Communication among Oral Bacteria

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INTRODUCTION

Communication is a key element in successful organizations. The bacteria on human teeth and oral mucosa have developed the means by which to communicate and thereby form successful organizations. These bacteria have coevolved with their host to establish a highly sophisticated relationship in which both pathogenic and mutualistic bacteria coexist in homeostasis. The fact that human oral bacteria are not found outside the mouth except as pathogens elsewhere in the body (51) points to the importance of this relationship. Communication among microorganisms is essential for initial colonization and subsequent biofilm formation on the enamel surfaces of teeth and requires physical contact between colonizing bacteria and between the bacteria and their host. Without retention on the tooth surface, the bacteria are swallowed with the saliva. Through retention, these bacteria can form organized, intimate, multispecies communities referred to as dental plaque.

Sequential changes in populations of bacteria associated with tooth eruption (20, 21, 102, 138) as well as with caries development (53) and periodontal disease states (109, 136) are known. Temporal changes in populations of bacteria on tooth surfaces after professional cleaning are ordered and sequential (92, 114, 115). Such sequential changes must occur through attachment and growth of different bacterial species. With the attachment of each new cell type, a nascent surface is presented for the attachment of other kinds of bacteria, resulting in a progression of nascent surfaces and concomitant changes in species diversity (79, 137). Such coordination indicates communication. In the absence of communication, these orderly changes would be random. Due to the dynamics of growth and adherence, the bacterial populations in the oral cavity are constantly changing, even during the intervals between normal daily oral hygiene treatments. It is unlikely that the various species within oral biofilms function as independent, discrete constituents; rather, these organisms function as a coordinated community that uses intra- and interspecies communication.

For the past 40 years, pure cultures of oral bacteria have been isolated from supragingival and subgingival dental plaque removed from healthy and diseased sites. The numbers and variety of bacteria obtained from many clinical conditions have been catalogued (10, 19, 72, 109, 137). Estimates of the bacterial species diversity in the oral cavity, based on both culture-dependent methods (109, 136) and culture-independent methods (83, 123), indicate about 500 species. About 415 species are estimated to be present in subgingival plaque (123), and many of these are also found in supragingival plaque. Cultured species account for about 60% of the organisms identified by molecular methods, indicating that the oral cavity is an environment where most species can be studied by routine culture methods. This is distinct from other environments where less than 1% of the clones obtained by molecular methods represent cultured species (2, 44, 54, 118). Thus, dental plaque is one of the best-described mixed-species bacterial communities. Because it is easily accessible, it is convenient to study this complex model system.

Most of the cultured species of oral bacteria have been tested for their ability to physically interact with and adhere to different species, and all display specific recognition patterns with their respective partner cells (154). This recognition between genetically distinct cells in suspension and resultant clumping is called coaggregation (72). Recognition between a suspended cell type and one already attached to a substratum is termed coadhesion (9). These interactions often appear to

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be mediated by complementary protein-adhesin and saccharide-receptor components on the two cell types (22, 27, 28, 143, 154). In many coaggregates, galactosides are competitive inhibitors of coaggregation, indicating a likely lectin-carbohydrate recognition between cognate molecules on the two cell surfaces (27, 78, 103, 154). These properties offer microbial ecologists and molecular biologists opportunities to explore the mechanisms of cell-cell recognition and their role in fostering bacterial biofilm communities in dental plaque.

Model systems useful for the study of mixed-species communities include biofilms on substrata (31, 155, 156) and planktonic communities grown in chemostats (11, 12). One model that has been employed to study oral bacterial colonization in vivo is a retrievable enamel chip worn by human volunteers (92, 115, 121). An in vitro model consisting of a flowcell with saliva-coated surfaces has offered an excellent platform for studying the adherence and growth of oral bacteria (75, 76, 119, 120). Community organization in the in vivo enamel chip and the in vitro flowcell model systems can be investigated by using confocal laser microscopy.

Avenues of communication among these ever-changing populations are likely to include metabolite exchange (57), cell-cell recognition (73, 120), genetic exchange (89), and signaling molecules produced by the host (82) and by other bacteria (15, 26, 48). A concert of communication methods probably occurs to establish transitory community organizations. The species participating in these oral communities compete and cooperate en route to establishing a climax community that may encompass all of the more than 500 known species. Overall, little is known about the communication mechanisms used by oral bacteria to establish these communities and to prevent disappearance from their habitat. This review focuses on these mechanisms, with the greatest attention devoted to cell-cell recognition and autoinducer-2, a signaling molecule produced by many bacteria, including oral bacteria. This review does not cover the equally exciting area of bacterium-host interactions.

In this review, our definition of communication among oral bacteria is not limited to effects of transcriptional activators, chemotactic signals, or two-component regulatory elements. Rather, here we include the view of bacteria responding to their environment. The first response in a biofilm is attachment to a surface, and this is communication. If adherence were nonspecific, then all bacteria could attach to oral surfaces, and this does not occur. After adherence, bacteria form multispecies communities. As in nonmicrobial ecosystems, some species are more successful at the beginning and other species are more successful at the end of community evolution; the timing of these successful opportunities may well relate to sequential modifications of the environment by the community. The methods by which these bacteria communicate to accomplish ordered multispecies communities is an active area of research, and we discuss some of the progress made in understanding communication among oral bacteria.

SPATIOTEMPORAL MODEL OF ORAL BACTERIAL COLONIZATION

Development of the oral microbial community involves competition as well as cooperation among the 500 species that compose this community. A few of those oral species are shown in Fig. 1 in a diagram illustrating competition and cooperation among early and late colonizers of the tooth surface. The acquired pellicle, which is composed of a variety of host-derived molecules, coats the enamel surface within minutes after professional cleaning and is a source of receptors recognized by the primary colonizers of dental plaque. These receptors include mucins, agglutinins, proline-rich proteins, phosphate-rich proteins such as statherin, and enzymes such as alpha-amylase (Fig. 1, blue-green columns, bottom). Each is a known receptor for particular oral species.

Streptococci constitute 60 to 90% of the bacteria that colonize the teeth in the first 4 h after professional cleaning (115). Other early colonizers include *Actinomyces* spp., *Capnocytophaga* spp., *Eikenella* spp., *Haemophilus* spp., *Prevotella* spp., *Propionibacterium* spp., and *Veillonella* spp. Many of the physical interactions that occur between the organisms of this community are known (72, 73, 154), and some are depicted in Fig. 1. The complementary symbols depict physical interactions known to occur between a pair of species. The different shapes and colors of the complementary symbols in Fig. 1 represent potentially distinct coaggregations. Rectangle-shaped symbols of any color represent lactose-inhibitable coaggregations, which are prevalent among oral bacteria (27, 72). Those of the same color represent functionally similar but not identical coaggregations. For example, the red rectangle symbols on the purple circle representing *Streptococcus oralis* and *Streptococcus sanguis* indicate a receptor polysaccharide named 1 Gn with the structure $\rightarrow PO_4^- \rightarrow$6GalNAcα1→3Rhaβ1→4Glcβ1→6Gal*f*β1→6GalNAcβ1→3Galα1→$)_n$ (28). The GalNAcβ1→3Gal receptor site in 1 Gn is recognized by functionally similar adhesins on *Streptococcus gordonii*, *Haemophilus parainfluenzae*, *Prevotella loescheii*, *Veillonella atypica*, *Eikenella corrodens*, and *Actinomyces naeslundii*. These adhesins are of various molecular sizes, and the species bearing the adhesins compete with each other for binding to the receptor polysaccharide (72, 73, 154). Thus, it is postulated that coaggregation and coadherence are integral to communication between species and help to establish patterns of spatiotemporal development.

Early Colonizers

Viridans streptococci (Fig. 1, bottom, purple circles), especially *S. gordonii*, are ideal model organisms to study because they are early colonizers, they coaggregate with a variety of oral bacteria, they bind to several host molecules, and many are genetically transformable. *Streptococcus* is the only genus of oral bacteria that demonstrates extensive intrageneric coaggregation as well as intergeneric coaggregation (73, 78). The ability to bind to other early colonizers and to host molecules (Fig. 1, lower half) may confer an advantage on these viridans streptococci in establishing early dental plaque. *S. gordonii* binds to salivary agglutinin glycoproteins by SspA/B (37), to alpha-amylase by AbpA (14, 132), and to the α2-3-linked sialic acid termini of O-linked oligosaccharides of host glycoconjugates by Hsa (142). Hsa has 113 serine-rich dodecapeptide repeats (142) and may be the sialic acid-binding adhesin involved in utilization of sialic acid as a nutrient, as has been shown for several viridans streptococci (17).

S. gordonii binds to acidic proline-rich proteins (PRPs) via the ProGln termini of PRPs (52). Acidic PRPs account for 25

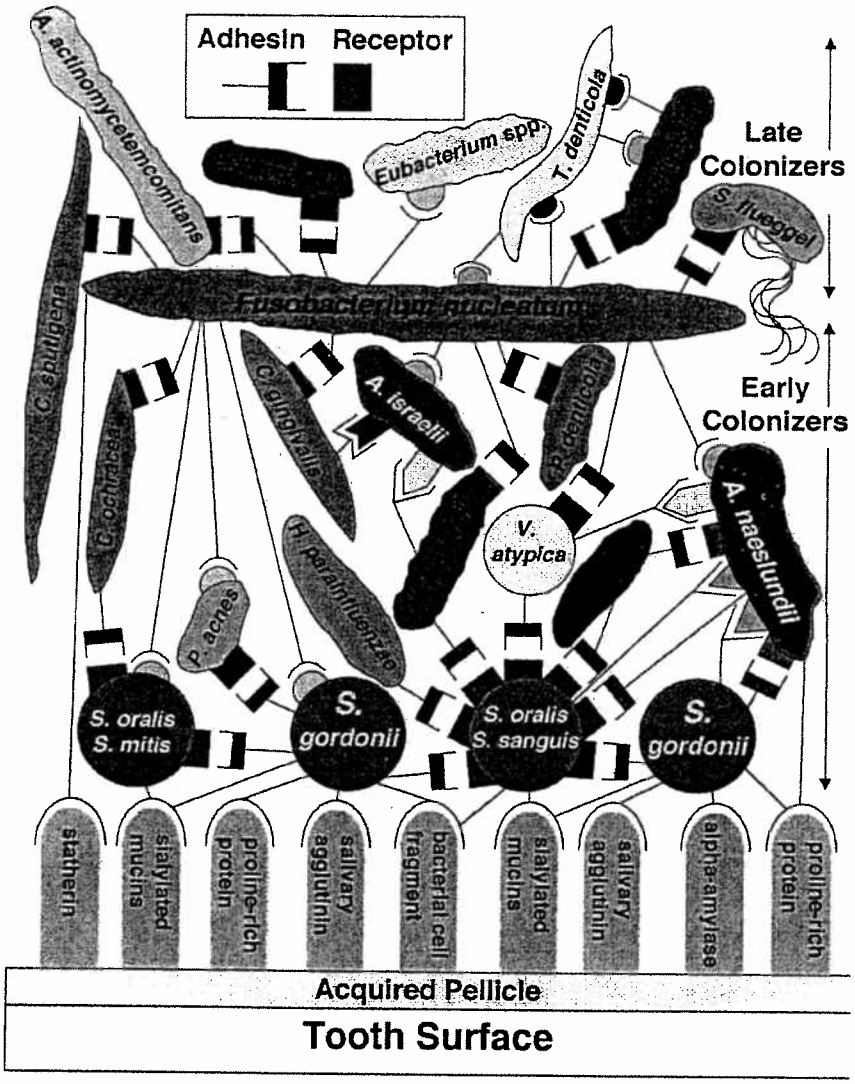

FIG. 1. Spatiotemporal model of oral bacterial colonization, showing recognition of salivary pellicle receptors by early colonizing bacteria and coaggregations between early colonizers, fusobacteria, and late colonizers of the tooth surface. Each coaggregation depicted is known to occur in a pairwise test. Collectively, these interactions are proposed to represent development of dental plaque and are redrawn from Kolenbrander and London (79). Starting at the bottom, primary colonizers bind via adhesins (round-tipped black line symbols) to complementary salivary receptors (blue-green vertical round-topped columns) in the acquired pellicle coating the tooth surface. Secondary colonizers bind to previously bound bacteria. Sequential binding results in the appearance of nascent surfaces that bridge with the next coaggregating partner cell. Several kinds of coaggregations are shown as complementary sets of symbols of different shapes. One set is depicted in the box at the top. Proposed adhesins (symbols with a stem) represent cell surface components that are heat inactivated (cell suspension heated to 85°C for 30 min) and protease sensitive; their complementary receptors (symbols without a stem) are unaffected by heat or protease. Identical symbols represent components that are functionally similar but may not be structurally identical. Rectangular symbols represent lactose-inhibitable coaggregations. Other symbols represent components that have no known inhibitor. The bacterial strains shown are *Actinobacillus actinomycetemcomitans, Actinomyces israelii, Actinomyces naeslundii, Capnocytophaga gingivalis, Capnocytophaga ochracea, Capnocytophaga sputigena, Eikenella corrodens, Eubacterium* spp., *Fusobacterium nucleatum, Haemophilus parainfluenzae, Porphyromonas gingivalis, Prevotella denticola, Prevotella intermedia, Prevotella loescheii, Propionibacterium acnes, Selenomonas flueggei, Streptococcus gordonii, Streptococcus mitis, Streptococcus oralis, Streptococcus sanguis, Treponema* spp., *and Veillonella atypica.*

to 30% of the total proteins in saliva; they regulate calcium phosphate and hydroxyapatite crystal equilibrium and thus contribute to stabilizing tooth integrity. They are encoded by host loci *PRH1* and *PRH2* and comprise five variants of 150 or 171 residues (63). One of these, acidic PRP-1, has been studied in some detail and may serve as a nutrient source for early colonizers. *S. gordonii* rapidly degrades the 150-amino-acid protein into two detectable peptides, the 105-amino-acid peptide derived from the amino-terminal region and a peptide corresponding to the 40 C-terminal amino acids, which are further degraded to oligopeptides (88). The pentapeptide expected to result from the intervening region was not detected,

but a synthetic peptide of the same sequence ($Arg_{106}Gly_{107}Arg_{108}Pro_{109}Gln_{110}$) caused detachment of *A. naeslundii* from PRP-1-coated latex beads, suggesting a mechanism for competition with other early colonizers (88). Thus, PRPs are not only receptors for binding bacteria (Fig. 1), they are also a ready nutrient source with an ecological bonus of modulating the adherence of potentially competing bacteria such as *A. naeslundii* (Fig. 1, lower right) at the tooth surface.

Oral bacteria capable of binding to a receptor and subsequently utilizing the receptor as a nutrient are suited to this task because they have diverse mechanisms for attachment. They can bind to other host receptors or to other bacteria while degrading the PRP nutrient. The numerous interactions between streptococci, host molecules, and other early colonizers demonstrate multifactorial communication between bacteria and their environment.

Early-colonizing bacteria have been shown to regulate gene expression in response to a saliva-containing environment. In saliva, *S. gordonii* DL1 increases expression of *sspA/B* (39), encoding surface proteins that bind to salivary agglutinin (37), an interaction depicted at the bottom of Fig. 1. Thus, streptococci suspended in saliva may bind to salivary agglutinin and elicit enhanced expression of *sspA/B*. Several other saliva-regulated genes include *S. gordonii hppA*, encoding an oligopeptide-binding lipoprotein, a homologue of the *Streptococcus mitis* glucose kinase gene *gki*, and a homologue of *Lactococcus lactis clpE*, encoding a member of the Clp protease family (39). These saliva-regulated proteins represent only a small assortment of the expected total proteins regulated by environmental conditions. They do, however, indicate that *S. gordonii* responds, at the transcriptional level, to elements from its natural oral environment.

Considering that each of the approximately 500 species in the oral bacterial community (Fig. 1) has such a potential for gene regulation in response to host-produced molecules and physical interactions with other bacteria, the complexity of possible interactions within the oral environment and the number of opportunities for cell-to-cell communication become daunting.

F. nucleatum and Late Colonizers

The bacterial species shown in Fig. 1 are placed in either of two general categories, early colonizers or late colonizers. This placement is based on the species of bacteria identified in dental plaque during temporal sampling after oral hygiene procedures were conducted (109, 115, 137). *Fusobacterium nucleatum*, however, is unusual and is intentionally placed at the border between early and late colonizers (Fig. 1) for the following reasons. First, *F. nucleatum* is the most numerous gram-negative species in healthy sites, and its numbers increase markedly in periodontally diseased sites (109). It is always present whenever *Treponema denticola* and *Porphyromonas gingivalis* are also present, suggesting that its presence predates that of the other two species and may be required for their colonization (137). Second, *F. nucleatum* coaggregates with all of the early colonizers and the late colonizers (4, 77). The bacteria representing early colonizers coaggregate with only a specific set of other early colonizers but not with all of them and generally not with any of the late colonizers (73, 79, 154). Although all the late colonizers coaggregate with *F. nucleatum*, they generally do not coaggregate with each other. A few exceptions, such as *T. denticola* coaggregating with *P. gingivalis*, have been reported (158) (Fig. 1, top right). Thus, *F. nucleatum* acts as a bridge between early and late colonizers, which may partially explain why fusobacteria are so numerous in samples from both healthy and diseased sites.

The cognate receptors (Fig. 1, blue rectangles) on the surface of the partners of *F. nucleatum* display functional similarity. They are depicted as competing for binding with the same *F. nucleatum* adhesins (Fig. 1, upper half). This idea is based on the observation that spontaneous mutants of *F. nucleatum* which were selected solely on the basis of being unable to coaggregate with *P. gingivalis* had also lost the ability to coaggregate with all of the lactose-inhibitable coaggregation partners but retained the ability to coaggregate with other partners (4). Just as these receptors exhibit functional similarity, adhesins recognizing the same 1 Gn receptor polysaccharide on *S. oralis* and *S. sanguis* (Fig. 1, lower half) exhibit functional similarity. The lactose-inhibitable coaggregations (Fig. 1, blue rectangles) between *F. nucleatum* and its partners appear to be mediated by the same galactose-binding adhesin that mediates attachment of *F. nucleatum* to mammalian cells, including human buccal epithelial cells and gingival and periodontal ligament fibroblasts (152). A mutant of *F. nucleatum* with no galactose-binding activity does not bind to eukaryotic cells. An independent study reported galactose-inhibitable binding and invasion of human gingival epithelial cells by *F. nucleatum* (59). These results attribute great potential significance to galactose-sensitive adhesins for initiating communication between early and late colonizers as well as with their host.

Finally, in addition to interactions with oral bacteria and host cells, *F. nucleatum* interacts with and binds host-derived molecules, such as plasminogen (33). *F. nucleatum* is generally nonproteolytic, but organisms that coexist with it, such as *P. gingivalis*, are highly proteolytic and can activate fusobacterium-bound plasminogen to form fusobacterium-bound plasmin, a plasma serine protease (33). Acquisition of proteolytic ability on its cell surface confers on the fusobacteria a new metabolic property, the ability to process potential peptide signals in the community. These peptides may be used as nutrients by fusobacteria or by other biofilm residents. *F. nucleatum* also induces expression of β-defensin 2, a small cationic peptide produced by mucosal epithelial cells (82). *P. gingivalis* does not elicit β-defensin 2 production, suggesting a distinction between these two important oral bacteria and their role in stimulating innate immune responses (82). Although *F. nucleatum* is often considered a periodontal pathogen, it may instead contribute to maintaining homeostasis and improving host defense against true pathogens.

Cooperation and Competition

In addition to fusobacteria acting as the principal coaggregation bridge between early and late colonizers, bridging among early colonizers is also possible. Some of these coaggregation bridges are shown in Fig. 1. For example, coaggregation between *P. loescheii* and *S. oralis* is lactose inhibitable (Fig. 1, red rectangle), and coaggregation between *P. loescheii* and *Actinomyces israelii* is lactose noninhibitable (Fig. 1, yellow

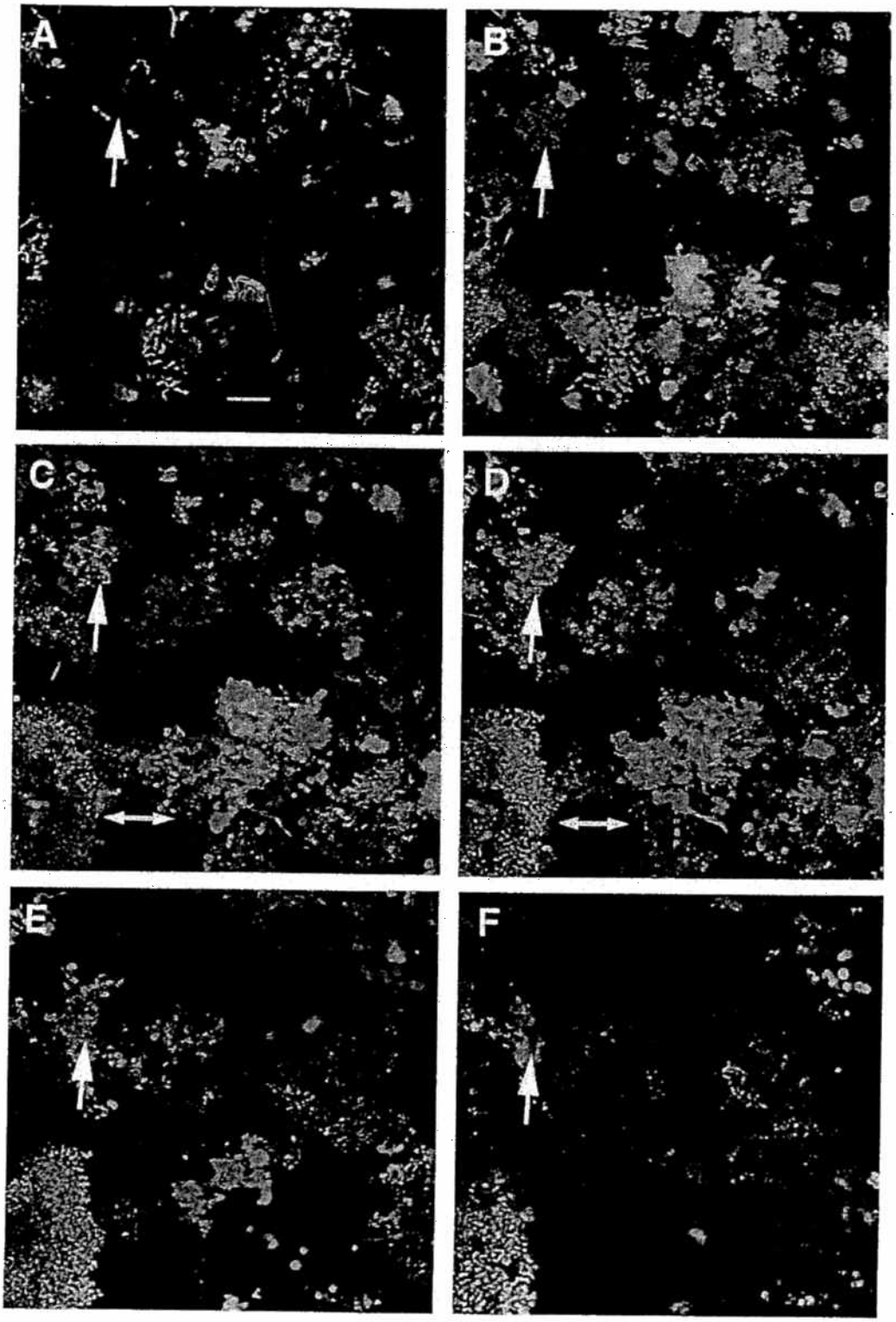

FIG. 2. Human oral biofilm formed in vitro with a saliva inoculum and using sterile saliva as its sole source of nutrient. The 25-μm-thick biofilm was grown overnight suspended from the underside of the coverslip of a flowcell with saliva flowing through once at 0.2 ml per min. Bacterial juxtaposition and biofilm architecture were imaged by confocal scanning laser microscopy after staining the cells with Live/Dead stain (Molecular Probes, Eugene, Oreg.). The color of the cells is from the red (propidium iodide; damaged or permeable cell membrane) and green (SYTO 9;

obelisk). *S. oralis* is not able to coaggregate with *A. israelii*; therefore, *P. loescheii* acts as a bridge of coaggregation. Both *A. israelii* and *P. loescheii* coaggregate with *F. nucleatum*, which coaggregates with all the late colonizers.

Coaggregation bridges are mechanisms of cooperation because they bring together two species that are not coaggregation partners. Such bridges may be critical for temporary retention of bacteria on a nascent surface and may facilitate eventual bacterial colonization of the biofilm. The bridges are distinct from competition, which occurs when multiple species compete for binding to the same receptor. Competitive and cooperative mechanisms may be central to successful mixed-species colonization as well as the proper succession of genera known to occur on teeth in both health and disease.

COMMUNITY ARCHITECTURE AND METABOLIC COMMUNICATION

In Vitro

Experimental approaches towards understanding human oral communities often start with in vitro studies and simple model systems. Several model biofilm systems have been developed, and most involve the flow of nutrients over a surface to which bacteria are attached (155, 156). Others employ a static support, such as a hydroxyapatite disk immersed in growth medium with gentle shaking (58). The substratum, nutrient medium, and oral bacteria chosen for study vary considerably among these models. Results from each model system contribute to our understanding of the growth and activity of bacteria on oral surfaces.

One system used in our laboratory is the flowcell, based on a design by Palmer and Caldwell (119), in which a microscope slide and coverslip are separated by a silicone rubber gasket that forms two channels (75). Sterile saliva is used as the sole nutrient source and is used to coat the glass before addition of bacteria to the flowcell. During a static period, bacteria bind to the saliva conditioning film, after which saliva flow is initiated, leading to the formation of a biofilm.

Inoculation of the flowcell with whole, unfiltered saliva followed by laminar flow of sterile saliva overnight results in a bacterial biofilm community attached to the saliva-coated substratum and extending towards the lumen (Fig. 2). After 18 h of growth, microcolonies consisting of different bacterial morphotypes are found in juxtaposition, suggesting construction of mixed-species communities. The spaces between these microcolonies indicate that communication by diffusible small molecules would require transmission over large distances and through a large volume of saliva compared to the cell-to-cell distances within a microcolony. The presence of several distinct cellular morphologies at the substratum (Fig. 2A) clearly indicates that many of the attached cells are already arranged as mixed-species communities.

Many initial attachments occur by only a few cells (Fig. 2A, arrow), compared to the contiguous, more voluminous colony mass extending toward the lumen (Fig. 2B, C, D, E, and F, arrows). This feature is characteristic of multispecies biofilm growth in laminar flow conditions as used in Fig. 2 and in certain monospecies biofilms (119). Under these conditions, initial colonization on the substratum is followed by axial growth and accumulation toward the lumen. In laminar flow, little shear force occurs at the substratum because salivary flow velocity is greatly reduced at the substratum compared to that in the lumen bulk phase. Accordingly, delivery of nutrient is higher in the faster-flowing region and may account for the greater biovolume found distant from the substratum. The larger cell masses may contact each other (Fig. 2C, double-headed arrow) and thus facilitate communication within the large surrounding void. Increasing separation between contacted communities (Fig. 2D, double-headed arrow) may accompany progression of the biofilm towards the lumen (Fig. 2E and F).

The apparent diversity and range of interactions that seem to occur in mixed-species communities (as shown in Fig. 2) help explain why seemingly healthy and active multispecies communities are capable of growth solely on saliva (34). The participants in this multispecies biofilm must signal each other in numerous ways from initial colonization through the various physiological stages en route to a mature biofilm community. It is important to consider that the laterally and axially (from top to bottom) organized communities are not composed of cells exhibiting just one cellular morphology. Rather, each of these adjacent communities may comprise many species. In fact, cells of nearly identical morphology may be different species.

Because of this complexity, such communities are difficult to study without highly specific tools or probes. Probes that are fluorescent permit imaging by confocal laser microscopy and can be used to examine spatiotemporal relationships. Several types have been used with success, including green fluorescent protein (5, 60), fluorescently labeled antibodies to surface antigens (5, 75, 120), and fluorescently labeled 16S rRNA-targeted probes (110).

Each bacterial species has a unique DNA sequence encoding the 16S subunit rRNA (16S rDNA). Modern bacterial phylogenetic schemes are based on the 16S rDNA sequence, and signature sequences unique to a particular species can be used as nucleotide probes for fluorescence in situ hybridization (FISH) to localize species in their natural habitat. Probe specificity can be evaluated by blot surveys against RNA from a range of oral species (122) or by mixing a selected group of oral bacterial species and examining by FISH the specificity of the

healthy cell) fluorescent stains. Colocalization of both fluorophores results in yellow staining. Confocal scanning laser microscopy acquires optical sections through the biofilm; each optical section is 0.5 μm thick. The entire biofilm is represented in six images (A to F). Panel A is the 0.5-μm optical section at the substratum and shows the biofilm footprint. Panel F is the top 0.5 μm of the biofilm where it projects into the lumen of the flowcell. The other four projection images contain eight sections per projection and show the 4-μm-thick regions from 4 to 8 μm from the substratum (B), 8 to 12 μm from the substratum (C), 12 to 16 μm from the substratum (D), and 16 to 20 μm from the substratum (E). Regions indicated by arrows are described in the text. Bar, 10 μm. Microscopic observations and image acquisition were performed on a TCS 4D system (Leica Lasertechnik GmbH, Heidelberg, Germany).

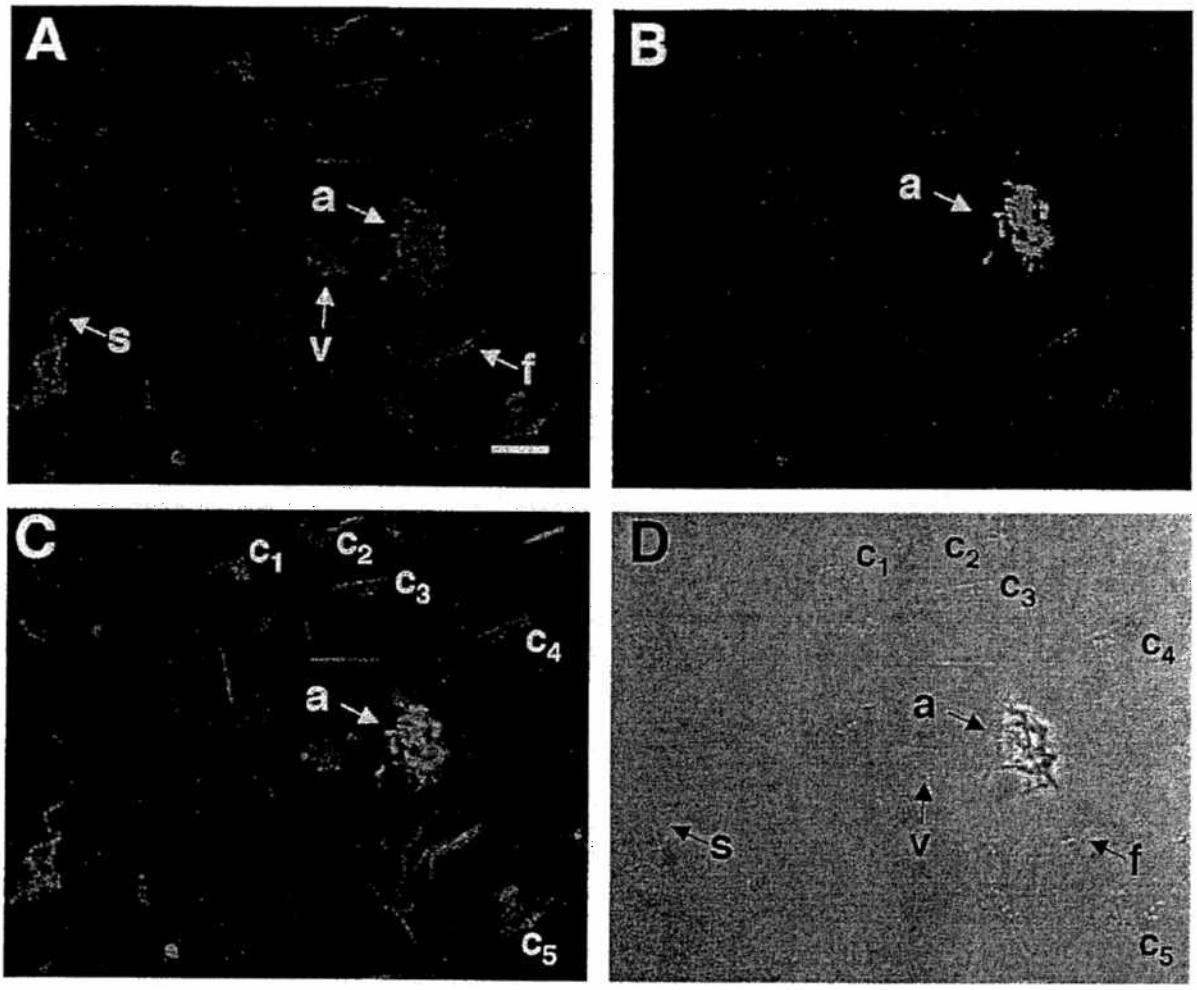

FIG. 3. Four-genus mixture of oral bacteria stained with a specific probe by FISH and the nonspecific nucleic acid stain SYTO 59. (A) Confocal micrograph of a field of cells stained with the nucleic acid stain SYTO 59. The field of cells contains the following species: *Actinomyces* serovar WVA963 strain PK1259 (a), *F. nucleatum* PK1594 (f), *S. gordonii* DL1 (s), and *Veillonella atypica* PK1910 (v). (B) Confocal micrograph of the same field showing the location of the fluorescein isothiocyanate-labeled actinomyces-specific probe. The image demonstrates that the probe interacts only with actinomyces cells (a). (C) Overlay of confocal micrographs (A and B), demonstrating the specificity of the actinomyces probe. Areas of colocalization of fluorescein isothiocyanate and SYTO-59 markers appear yellow. The yellow actinomyces cells are in contact with other cells seen at the edges of the actinomyces cluster. Coaggregations (c_1 to c_5) of different species within the mixed culture are also visible. (D) Differential interference contrast image of the field of cells using transmitted light. The distinct morphologies of the various cells in the mixed culture are visible. Bar, 10 μm. Microscopic observations and image acquisition were performed on a TCS 4D system (Leica Lasertechnik GmbH, Heidelberg, Germany).

probe in whole cells by confocal microscopy (Fig. 3). In Fig. 3, the probe was targeted to the 16S rRNA of *Actinomyces* serovar WVA963 strain PK1259 and was specific for actinomyces cells (Fig. 3B, green cells). This mixture of cells also contained fusobacteria (spindle-shaped cells), streptococci (spherical cells in chains), and veillonellae (smaller spherical cells in clumps). Four oral bacterial genera are represented here, and each coaggregates with the other three.

Several coaggregates were visible after briefly mixing the four species to prepare this specimen (Fig. 3). The large coaggregate (Fig. 3C, arrow), consisting of actinomyces (yellow) and other cells (red), clearly shows the juxtapositioning of distinct cell types. Juxtapositioning is also seen in the examples of other coaggregations (Fig. 3C and D, $c_{1\text{-}5}$), but the identity of the spherical cells cannot be unequivocally determined from morphology alone. The specificity of nucleic acid probes makes them particularly useful in identifying bacteria in situ, and FISH would be an excellent method for identifying the organisms shown in Fig. 2. Thus, specific 16S rRNA-targeted probes are useful for identifying oral bacteria within dental plaque without disrupting community architecture. The combination of FISH with autoradiography can provide additional information on the metabolism of these cells in complex communities by coupling identification of species with uptake of radiolabeled substrates (87, 117).

Using fluorescently conjugated antibodies raised against cell